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MTHFR shRNA (h) Lentiviral Particles: sc-43950-V

BACKGROUND

5,10-methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the folate-dependent regulation of methionine and homocysteine concentrations. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate (methyleneTHF) to 5-methyltetrahydrofolate (methylTHF). MethylTHF, the predominant form of circulating folate, is the principle carbon donor for homocysteine methylation, a reaction that yields methionine. Folate prevents neural tube defects, and can lower homocysteine levels, suggesting that MTHFR function is important in preventing cardiovascular disease. Mutations at the MTHFR gene may influence vascular changes, predisposition to leukemia, coronary artery disease and hyperhomocysteinemia.

REFERENCES

1. Homberger, A., et al. 2000. Genomic structure and transcript variants of the human methylenetetrahydrofolate reductase gene. *Eur. J. Hum. Genet.* 8: 725-729.
2. Lucock, M. 2000. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol. Genet. Metab.* 71: 121-138.
3. Gaughan, D.J., et al. 2000. The human and mouse methylenetetrahydrofolate reductase (MTHFR) genes: genomic organization, mRNA structure and linkage to the CLCN6 gene. *Gene* 257: 279-289.
4. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 236250. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. LocusLink Report (LocusID: 4524). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: MTHFR (human) mapping to 1p36.22.

PRODUCT

MTHFR shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see MTHFR siRNA (h): sc-43950 and MTHFR shRNA Plasmid (h): sc-43950-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

MTHFR shRNA (h) Lentiviral Particles is recommended for the inhibition of MTHFR expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

MTHFR (N-20): sc-17079 is recommended as a control antibody for monitoring of MTHFR gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MTHFR gene expression knockdown using RT-PCR Primer: MTHFR (h)-PR: sc-43950-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.